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6. AUTHOR(S)
Michael Syvanen

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of California at Davis
School of Medicine, One Shields Avenue
Davis, CA 95616-8645

8. PERFORMING ORGANIZATION REPORT NUMBER

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13. ABSTRACT (Maximum 200 words)

Glutathione transferases are ubiquitous in nature. So far they have been found in all eukaryotes and most bacteria strongly implying they serve an essential function for life. This function appears to be no more than an aid to organisms to detoxify certain chemically active agents that can be toxic. In the housefly *Musca domesitica* we have been studying the gst gene and its encoded enzyme that is responsible for detoxifying the organophosphotriester neurtoxins (OP) that are used as insecticides. The gene designated gst6a is one of over a dozen found in the organism, has been cloned and expressed in *E.coli*. In addition, we have found a role for *E.coli*'s native gst gene; it provides protection against the toxic effects against certain halogenated alkanes. One of these compounds, 1,2 dibromo ethane, is of interest as an industrial pollutant and thus, there is a potential bioremediation role for the gene. Regulation of the *E.coli* gst gene is complex and appears to be tied into one of more of the bacteria's stress response regulons.

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Summary of the most important results

There were two primary research projects carried out over the past three years under the ARO grant. These are the characterization of the housefly (*Musca domesica*) genes responsible for the detoxification of the organotriester phosphate neurotoxins (OP) and studies on a halogenated alkane detoxification system in bacteria. Both of these systems use glutathione transferase (GST) as the primary detoxification enzyme.

Housefly Glutathione Transterases

In earlier reports we have described the cloning, sequencing and partial mapping of over a dozen gst genes isolated from organophosphate resistant houseflies. In the latest period we have worked on identifying which of these dozen is the one primarily responsible for resistance in the housefly. Two approaches have been employed.

i. Large scale genomic mapping.

We have a series of housefly mutants that are altered in the gst responsible for OP resistance. We have used these mutants to guide the cloning of genomic copies of these gst genes (see Zhou et al 1995 for initial efforts). Early studies showed that the gst OP resistance genes were members of very large genomic amplifications. Most recently we have embarked upon constructing very large genomic clones. Technically this involves building what is known as a housefly "BAC library". This is an involved time consuming process. So far we have isolated fragments of the housefly genome in the 100-200 kilo base size range. In addition, we have purified large amounts of a

single copy plasmid vector that forms the replicon for the BAC clones. We have also worked out the procedures for inserting, by transformation, completed BAC clones into the recipient bacteria. The next stage will involve scaling up our production of BAC clones so that we can create the needed 50,000 clones that will cover the whole housefly genome. This work is in progress.

ii *Counting genes using kinetic PCR.*

One of my colleagues here at UC Davis, Michael Holland, has helped perfect a developing technology that is beginning to prove very powerful as a quantitative tool for DNA and RNA research. This is an application of kinetic PCR and has much greater sensitivity and accuracy than the older technique that rely on nucleic acid hybridization (i.e. Northerns, Southerns, Dot Blots, Micro Chips, etc.). We have applied this procedure to counting gst genes in the housefly. The results have been striking. These are described in the abstract to the ARO Northampton Conference held in 2001 (abstract enclosed). The basic result is that only one of our gst genes is amplified in the OP resistant housefly; gst6A is present in about 100 copies per genome in resistant houseflies and in only 1-2 copies per genome in sensitive flies. Gst6A is described in the enclosed abstract reprint (Wei and Syvanen, 2001).

During the collaboration with Michael Holland on this kinetic PCR project, I also contributed ideas and did some bacterial projects. That work is described in the enclosed abstract (1 and 2) and the work is in preparation for three full length papers.

Bacterial Glutathione Transferase.

The second ongoing project supported directly by the ARO concerns the glutathione transferase found in the bacterium *Escherishchia coli*. We began this work to gain greater insight into the effects of expressing housefly gsts in this bacterium. The preliminary work has been described in earlier reports and at the High Hampton ARO workshop held in 1998. The most recent finding is that we have discovered a role for the gst gene in *E. coli*. We constructed a knockout of the gst gene which gave us an isogenic pair of strains that are gst+ and gst(-). The two strains grow identically in over 60 different environments that we tested except for two. The compounds 1-Iodo 2-methyl propane and 1,2 dibromo ethane are toxic to E. coli. We have found that the gst - strain is hypersensitive to these two compounds compared to the gst+ strains. Thus, we have found two compounds that are presumably detoxified by the bacterial enzyme.

These two halgenated alkanes are synthetic and presumably are not natural products. We do not think that *E. coli* evolved a gst to detoxify these compounds but this does add to the list of bacteria that have innate abilities to metabolize completely foreign chemicals and this enables them to be of use as agents to clean up human created pollutants.

In addition to the above projects the P.I. has been involved in other work in which resources provided through the ARO were used. The resulting publications are listed.

1998 **Syvanen, M**. and C. Kado, editors, Horizontal Gene Transfer, Chapman & Hall, London. 474 pgs.

1998 Turner, K.O., **M. Syvanen** and S. Meizel. The human acrosome reaction is highly sensitive to inhibition by cyclodiene insecticides. Journal of Andrology, 18:571-575.

1999 **Syvanen, M**. Temporal patterns of land plant evolution suggest extensive polyphyly. Endocytbiosis and Cell Research

1999 **Syvanen, M.** In search of horizontal gene transfer. Nature Biotechnology. 17 (9): 833

2000 Boucouche, N., **M. Syvanen** and C. Kado. The origin of prokaryotic C2H2 finger regulators. Trends in Microbiology. 8 (2): 77-81.

2001 **Syvanen, M.** and C. Kado, editors, Horizontal Gene Transfer, 2nd ed. Harcourt Press, London. 427 pgs.

2001 Wei, S. and **M. Syvanen.** Identification and cloning of a novel insecticide-metabolizing glutathione s-transferase (Md GST-6A) key to hyper resistance in the horsefly *Musca domesitica.* Insect Biochemistry and Molecular Biology. *See reprint.*

2001 **Syvanen, M.** On the occurrence of horizontal gene transfer among an arbitrarily chosen group of 26 genes. Journal of Molecular Evolution, *in press.*

2001 **Syvanen, M.** Emergence of the modern genetic code: a proposal. Trends in Genetics. *in press*

2001 **Syvanen, M.** Rates of ribosomal RNA are uniquely accelerated in eukaryotes. J. Mol. Ecol. *In press.*